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COMPLEX SYSTEMS
BIOPHYSICS

Protective Effect of Low-Intensity Laser Irradiation under Acute Toxic Stress

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Abstract—We studied how short-term preexposure of the thymus zone in male outbred NMRI mice to helium–neon laser light (632.8 nm, 0.2 mW/cm^2) affects the activity of cells of the immune system under acute toxic stress. The stress was modeled by introducing a bacterial lipopolysaccharide that significantly enhanced the production of a number of cytokines in macrophages: interleukins 1α, 1β, 6, and 10, and tumor necrosis factor TNF-α. Single exposure of healthy mice to laser light did not cause any significant change in the production of cytokines and nitric oxide in cells but increased the production of the heat shock proteins HSP25, HSP70, and HSP90. Nonetheless, if mice were exposed to red light before inducing toxic stress, then the production of almost all the cytokines studied and nitric oxide was noticeably normalized. Moreover, the production of the heat shock proteins studied was also normalized. Thus, preexposure of a small region of the animal skin surface to laser light markedly decreased the toxic effect of lipopolysaccharide.

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Key words: toxic stress, laser radiation, heat shock proteins, cytokines, nitric oxide

INTRODUCTION

We previously showed that low-intensity laser radiation can not only stimulate cells of the immune systems, but also, under certain conditions, can inhibit their activity. For example, the activity of natural killer cells, which are important components of the nonspecific immune defense, could be controlled by varying the exposure to low-intensity red light and the location of the exposed body region [1]. In this case, fractionated low-dose irradiation of the thymus zone over a long time suppressed the activity of natural killer cells, whereas irradiation of the hindlimb skin surface under the same conditions stimulated their activity. Similar cell responses to long-term in vivo exposure (every third day) to helium–neon laser light were detected in studying the production of cytokines, nitric oxide (NO), and heat shock proteins (HSP) in healthy animals [2]. Moreover, stimulation of the antitumor immunity was observed for several days after a single in vivo exposure to laser light [3]. Dose dependence of the activity of cells of the immune system has recently been demonstrated in low-intensity laser light irradiation of isolated cells of male outbred NMRI mice. For in vitro irradiation of a population of T cells and macrophages, we showed that doses not exceeding $6\cdot10^{-3}$ J/cm^2 stimulated the production of TNF-α, IL2, IL6, IFNγ, and NO and the activity of natural killer cells. An increase of the radiation dose led mainly to inhibition of the secretory activity of macrophages and T cells and also to suppression of the cytotoxic activity of natural killer cells [4]. Thus, on in vivo and in vitro exposure to weak laser light, a general tendency was noted: short-term irradiation

Abbreviations: LPS, lipopolysaccharide; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; HSP, heat shock proteins.

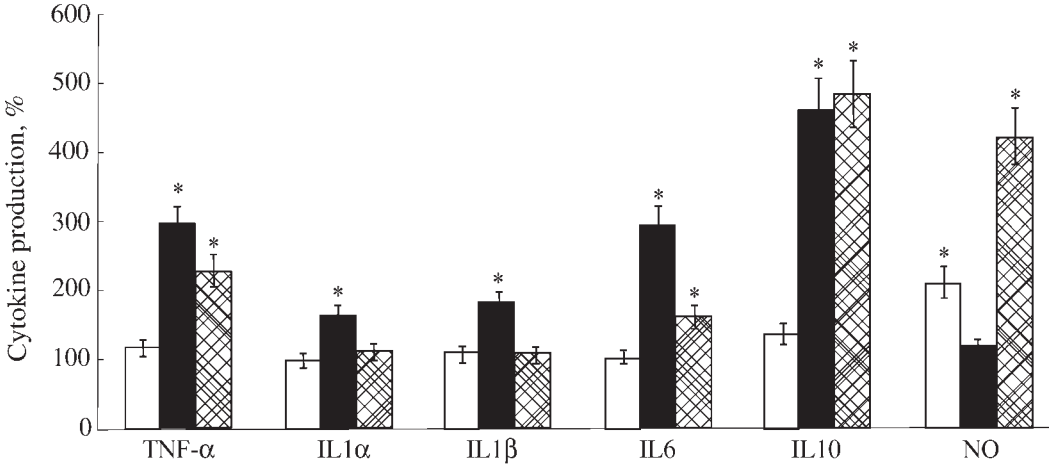

Fig. 1. Production of cytokines and NO in peritoneal macrophages of mice exposed to low-activity laser light before inducing toxic stress. The empty bins represent exposure to laser light; the filled bins, introduction of LPS; and the cross-hatched bins, exposure to laser light with subsequent introduction of LPS. The measurements were made in 6 h after introduction of LPS. Each value (percentage of control) is the mean of three to four independent experiments. All the measurements were made individually for each animal in sextuplicate. The controls were mock-irradiated mice with saline injections. *Reliable difference from control, $p < 0.05$.

primarily stimulated the secretory activity of cells, whereas an increase of the dose mostly had an immunosuppressive effect.

We have recently shown that, in mouse cells under acute toxic stress caused by introducing a bacterial toxin, the expression of HSP70 and HSP90α is enhanced and the production of TNF-α, TNF-β, and NO increases [5]. Moreover, the acute intoxication of the organism was accompanied by marked accumulation of pro-inflammatory cytokines in the peripheral blood. In the context of the previously detected imunomodulatory effect of low-intensity laser light, the purpose of this work was to study the regulation of toxic responses of the organism, using laser radiation as an efficient tool for affecting immunity.

EXPERIMENTAL

Experiments were performed on adult male outbred NMRI mice weighing 20–25 g. The source of red laser light was an LGN-111 helium–neon laser with $\lambda = 632.8$ nm and an incident radiation power of 0.2 mW/cm^2. The thymus zone was irradiated using a light guide for 1 min to a dose of $1.2 \cdot 10^{-2}$ J/cm^2 12 h before introducing endotoxin. In irradiation, the body of the animal was fixed and shielded with dense white paper having a 1-cm hole over the exposed region of shaved skin. Acute toxic stress was caused by a single intraperitoneal injection of 250 μg lipopolysaccharide from *Escherichia coli* (Serotype 026.B6, Sigma, USA) per 100 g body weight, and the animals were decapitated in 6 h after introduction of endotoxin. The controls were mock-irradiated mice injected with saline.

Isolation of peritoneal macrophages and T cells, immunoenzyme assay for cytokines, and determination of NO and HSP were performed as described previously [5]. The primary antibodies in the immunoenzyme assay were rabbit polyclonal antibodies against mouse TNF-α, IL1α, IL1β, IL6, IL10, and IFNγ; all the antibodies and cytokine proteins were from PeproTech (USA). The secondary antibodies were biotin-conjugated goat antirabbit IgG (StressGen, Canada). The production of HSP was measured using mouse monoclonal antibodies against HSP72 (clone SPA-812, inducible form), HSP25 (clone SPA-801), and HSP90α (clone SPA-828), all from StressGen.

Statistical processing of the results was performed using the Student's *t*-test.

RESULTS AND DISCUSSION

Production of Cytokines and Nitric Oxide in Macrophages

Figure 1 presents the production of cytokines and NO in macrophages from three groups of mice treated with laser light, lipopolysaccharide (LPS), or both. The single irradiation of the thymus zone caused no marked changes in the production of the cytokines studied, whereas the NO production reliably

increased. In macrophages from LPS-treated mice, the production of TNF-α, IL6, IL10, and NO sharply increased. The stimulatory, albeit not so efficient, action of LPS on the production of IL1α and IL1β was also revealed. If mice were exposed to laser light before intoxication, then the peaks of the cytokine-producing activity of macrophages were much lower. The production of IL1α and IL1β was completely normalized, and the peaks of TNF-α and IL6 reliably decreased.

The only exception was IL10, for which no modulatory effect of laser light was detected. Indeed, the production of IL10 abruptly increased after introduction of LPS regardless of laser irradiation. Since IL10 is known to have a pronounced anti-inflammatory effect, this also can be regarded as a beneficial effect of red laser light under acute intoxication.

Thus, a single preexposure of mice to laser light significantly decreased the effect of acute intoxication of animals by decreasing the production of pro-inflammatory cytokines in macrophages. Preexposure to laser light also considerably increased the peak of the production of reactive nitrogen species (NO) in the LPS-treated animals.

Production of Cytokines and Nitric Oxide in Spleen Lymphocytes

For another population of cytokine-producing cells, namely, spleen lymphocytes, we also detected a normalizing effect of preexposure to laser light on the production of IL2, IL10, and IFNγ (Fig. 2). A single exposure of healthy mice to laser light led to a slight stimulation of the production of IL2 and IL10 in spleen lymphocytes, whereas the acute intoxication of the animals caused a more significant stimulation of the production of IL2, IL10, and IFNγ in lymphocytes. Note that, under the joint action of the two factors, the production of cytokines considerably decreased and approached the control value. Thus, the effects of laser light and endotoxin on animals were not additive; conversely, preexposure decreased the toxic effects induced by LPS.

Expression of Heat Shock Proteins

The results showed that acute toxic stress caused a significant increase in the production of HSP, which was most pronounced for HSP25 and HSP70 (Fig. 3). On the other hand, a single exposure of the skin surface of healthy mice to low-intensity laser light also stimulated HSP production. Under the joint action of the two factors, each causing a stress response of cells, the effects were not additive. On the contrary, preexposure to laser light almost completely relieved the stress response of the cell to endotoxin and normalized the HSP production.

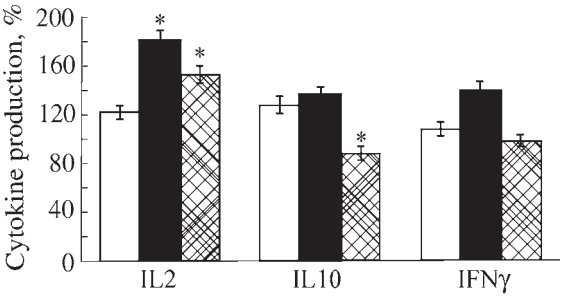

Fig. 2. Production of cytokines in spleen lymphocytes of mice exposed to low-intensity laser light before inducing toxic stress. Notation and explanations as in Fig. 1.

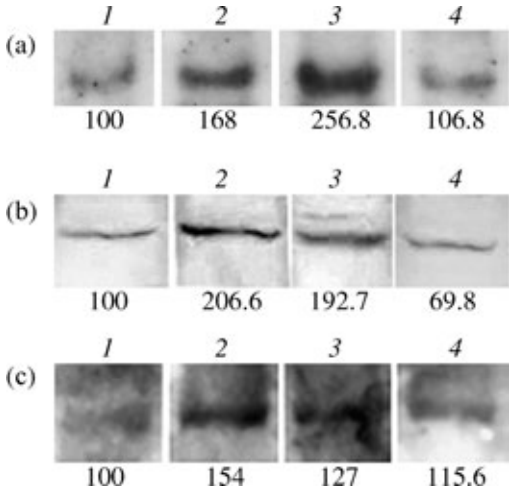

Fig. 3. Expression of (a) HSP25, (b) HSP70, and (c) HSP90 in spleen lymphocytes of mice exposed to laser light with subsequent introduction of LPS. The measurements were made in 6 h after LPS. The numbers under the strips of the membrane treated with antibodies represent the relative protein concentrations (percentage of control) calculated using the Qapa program. Panels *1* represent control samples; panels *2*, exposure to laser light; panels *3*, introduction of LPS; and panels *4*, exposure to laser light with subsequent introduction of LPS.

Thus, in this work, we proved the immunocorrecting activity of low-intensity laser light using a model of acute toxic stress causing a systemic disturbance of the functioning of immunocompetent cells. The mechanism of protective action of low-intensity laser light is probably associated with its stimulatory effect on cellular immunity. This property of laser radiation is used in various types of laser therapy for, e.g., tumors [6, 7], Parkinson's disease [8] and other

disorders [9, 10]. The results of this work proved that the preventive use of red laser light can significantly decrease the acuity of the toxic response of the organism to bacterial infection.

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